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MAST® ASSURE ANTISERUM CAMPYLOBACTER

Intended Use

Liquid stable antisera for the serotyping the heat stable antigens of *Campylobacter jejuni* by the passive haemagglutination procedure.

MAST® ASSURE REAGENTS FOR PREPARATION OF SENSITISED RED BLOOD CELLS

Reagents for extracting bacterial antigens and for coupling the extracted antigens to chick red blood cells. For use in the serotyping of C*ampylobacter jejuni* by the passive haemagglutination method.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents: See pack label.

Formulation

MAST® ASSURE ANTISERUM CAMPYLOBACTER are prepared from rabbits hyperimmunised with standard strains of killed organisms possessing known serotypes or group specific antigens and contain 0.085% sodium azide as preservative.

MAST® ASSURE REAGENTS FOR PREPARATION OF SENSITISED RED BLOOD CELLS contains the following components:

1. Fixed Chick Red Blood Cells Sample Diluent, ready to use. 1x 25ml. Contains aldehyde fixed chick erythrocytes suspended in saline.
2. Extraction Reagent 1, ready to use. 1x 13ml. Contains a solution of acetic acid.
3. Extraction Reagent 2, ready to use. 1x 13ml. Contains a solution of sodium nitrite.
4. Extraction Reagent 3, ready to use. 1x 13ml. Contains a Tris buffered solution.
5. Buffer Solution, ready to use. 2x 50ml. Contains a phosphate buffered saline solution.

Components 1, 4 and 5 contain 0.085% sodium azide as preservative.

Stability and storage

Store unopened at 2 to 8°C until the expiry date shown on the pack label. Once opened, MAST® ASSURE ANTISERUM should be stored at 2 to 8°C and may be used until the expiry date given on the label. **Do not freeze reagents.**

Warnings and precautions

For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.

Materials required but not provided

Standard microbiological supplies and equipment such as loops, applicator sticks, test tubes, pipettes, swabs, MAST® culture media, incinerators and incubators, etc., as well as specific items such as:

- sterile 0.85% saline solution
- centrifuge capable of achieving 7000 rpm.
- V-bottom microwell plates
- humid chamber
- MAST® ASSURE CAMPYLOBACTER ANTISERUM are designed for use in conjunction with MAST ASSURE REAGENTS FOR PREPARATION OF SENSITISED RED BLOOD CELLS. These reagents can be ordered as code M42201.
- MAST® ASSURE REAGENTS FOR PREPARATION OF SENSITISED RED BLOOD CELLS are provided for use with MAST® ASSURE CAMPYLOBACTER ANTISERUM.

Procedure

A. Extraction of Bacterial Antigens.

1. Dispense 0.25ml of sterile saline solution into a 1.5ml centrifuge tube.
2. Using a bacteriological loop, emulsify bacterial cells from the test culture in the saline (i.e. an amount about the size of a matchhead).
3. Add to the suspension 0.25ml each of Extraction Reagent 1 and 2 in sequence. Cap the tubes, mix the suspension using a vortex mixer and incubate at room temperature for 10 minutes.
4. After 10 minutes add 0.25ml of Extraction Reagent 3 and mix well.
5. Centrifuge the tube for 5 minutes at 7000rpm and use the supernatant as the bacterial antigen solution for sensitisation of the chick red blood cells.

B. Preparation of Chick Red Blood Cells.

1. Ensure that the fixed chick red blood cells are fully resuspended before use to a uniform homogeneity then dispense 0.5ml into the required number of test tubes.
2. Add 0.5ml of Buffer Solution and centrifuge the mixture at 3000rpm for 10 minutes.
3. Carefully discard the supernatant and resuspend the pellet in 0.5ml of buffer.

C. Preparation of Sensitised Chick Red Blood Cells.

1. Take a 1.5ml centrifuge tube and dispense 0.5ml of the bacterial antigen solution for sensitisation followed by 0.5ml of the prepared fixed chick red blood cells. Cap the tube and mix well, then incubate at 37°C for 30 minutes. During incubation agitate the tube at regular intervals.
   Note: Ensure that the fixed chick red blood cells are fully resuspended before use to a uniform homogeneity.
2. After 30 minutes incubation centrifuge the tube at 6000rpm for 30 seconds and carefully discard the supernatant.
3. Add 1.0ml of buffer and resuspend the pellet using a vortex mixer. This should now be considered as the sensitised cell suspension.

D. Passive Haemagglutination Procedure.

1. Dispense 1 drop of each antiserum into separate wells of a V-bottom microwell plate according to a pre-planned template. Also dispense 1 drop of the reference serum into an additional well as a control.
2. Dispense 25µl the sensitised cell suspension into each well. Allow the sensitised cell suspension to freefall into the well so as not to contaminate it with antisera.
3. Gently mix the contents of the wells using a microplate mixer or by hand, place in a humid chamber and incubate at room temperature for 30 to 60 minutes.
4. After 30 to 60 minutes check each well for agglutination and interpret the results according to the criteria listed below.

Interpretation of results

1. The control well containing reference serum should show negative agglutination i.e. the red blood cells (RBC) should form a tight button at the centre of the well only. If the reference serum fails to give a negative reaction it should be retested or the extraction of the bacterial antigen repeated.
2. Results for the individual typing antisera should be interpreted according to the table below.

Result	Pattern	Interpretation
RBC's sediment to form a tight button at the centre of the well.		-
RBC's show some agglutination but do not cover the surface of the well.		+
RBC's agglutinate to cover the complete surface of the well, but there is still a visible button of cells at the centre of the well.		++
Full agglutination of RBC with no visible button of cells at the centre of the well.		+++

3. A bacterial isolate producing a distinct positive reaction (+, ++ or +++) with an specified antiserum is assumed to be a strain of *Campylobacter jejuni* bearing antigen factors represented by that group antiserum. If the bacterial isolate produces a positive reaction with more than one antiserum it should be regarded as a complex type strain.

The antisera are labelled according to Penner's grouping and numbering system, as follows:

Group A: 1, 44	Group K: 12	Group Y: 37
Group B: 2	Group L: 15	Group Z: 38
Group C: 3	Group N: 18	GroupZ: 41
Group D: 4, 13,16, 43, 50	Group O: 19	Group Z: 45
Group E: 5	Group P: 21	Group Z: 52
Group F: 6, 7	Group R: 23, 36, 53	Group Z: 55
Group G: 8	Group S: 27	Group Z: 57
Group I: 10	Group U: 31	Reference
Group J: 11	Group V: 32	

Limitations of use

Only cultures of organisms isolated by conventional methods and confirmed as *Campylobacter jejuni* by morphological and biochemical features may be serotyped by this procedure. All *C jejuni* isolates should be grown on blood agar plates at 42°C for 48 hours under microaerophobic conditions prior to commencing the procedures.

Quality control

It is recommended that quality control should be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. Check for signs of deterioration. Do not use reagents if they are contaminated or cloudy.

References

Bibliography available on request.

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